REMARKS

Claim Amendment

Claims 17 and 38 has been amended to delete reference to "siRNA" in every instance where the term describes expression cassette.

Claim 33 has been amended by 1) adding the term "at least one" to the preamble, such that it reads, "[a]n amplification-based method for producing at least one mammalian promoter-containing siRNA expression cassette;" 2) deleting reference to "siRNA" in every instance where the term describes expression cassette; 3) specifying that the primer and expression cassette comprises the sense sequence of a double stranded siRNA molecule; 4) reciting "a first" amplified product; and 5) correcting an error by adding "primer" such that it reads, "the second oligonucleotide primer."

Claim 36 has been amended to recite additional method steps, i.e. steps (a) – (d) which are the same as in claim 33 except that the second oligonucleotide primer comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes an antisense sequence of the double stranded siRNA molecule to produce a second amplified product.

Claims 39 and 40 have been added, which mirror claims 33 and 36, respectively, except in the first amplification, the primer comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes an antisense sequence of the double stranded siRNA molecule and in the second amplification, the primer comprises a nucleotide sequence that is complementary to a nucleotide sequence that encodes a sense sequence of the double stranded siRNA molecule.

New claim 41 has been added which mirrors claim 17 but is dependent on new claim 40.

Support for these amendments can be found, for example, at Figure 1A (depicting two PCR amplifications to produce two PCR cassettes – one that expresses the sense siRNA and another that expresses the antisense siRNA) and paragraphs 13, 56, 63 (see reference to multiple "PCR products" that include "the siRNA sense or antisense encoding sequence").

Applicants submit that none of these amendments constitute new matter, and their entry is requested.

Objections

The Examiner objected to claims 17, 19-21, and 36-38 under 37 CFR 1.75(c), as being of improper dependent form for reciting additional method steps with additional resultant products than the method steps/product of claim 33, thereby failing to further limit the method steps and single product claimed in claim 33. Claim 33 is directed to "an" amplification-based method for producing "a" mammalian promoter-containing siRNA expression cassette and thus, requires a single PCR that produces a single cassette. Dependent claims 17, 19-21, and 36-38 require "two polymerase chain reaction amplifications," thereby producing two cassettes: "a first amplified product" and a "second amplified product."

The amendments to claims 17, 33, 36, 39 and 40 clarify that "an amplification-based method" is not limited to a single PCR amplification, that the expression cassette does not contain a double stranded siRNA, and that the claims directed to a second amplification are proper dependent claims that recite additional steps. Accordingly, withdrawal of this objection is requested.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 3-9, 17, 19-21, and 33-38 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner begins by erroneously stating that claim 33 is directed to "a method claim that produces double-stranded, short RNA fragments." Office Action, p. 3. The Examiner relies on claim language that describes "siRNA" as being "double stranded" (step iii) and the specification's disclosure that "siRNA" refers to double-stranded RNAs of 21 to 23 nucleotide (nt) fragments (paragraph 0004). The Examiner then characterizes "the intended product" as a single cassette that produces/encodes a double stranded siRNA. Office Action, p. 4, 5. The Examiner points out that there is no method step to synthesize both strands (sense strand and antisense strand). The Examiner refers to method step (iii) which recites a "second oligonucleotide primer" that hybridizes to a nucleotide sequence encoding "either a sense sequence of a double stranded siRNA molecule or an antisense

sequence of the double stranded siRNA molecule." According to the Examiner, the resultant product of the method is a cassette that produces only either one of the strands, rather than the intended product, "a cassette that produces a double-stranded siRNA." Office Action, p. 4. The Examiner further argues that claim 36, from which claims 17, 19-21, and 37-38 depend, is inconsistent with claim 33 because it requires two PCR methods and "a second amplified product"/ "two independent cassettes," while the intended product of claim 33 is a single siRNA expression cassette.

The amendments to claims 17, 33, 36, 39 and 40 clarify that "an amplification-based method" is not limited to a single PCR amplification, that the expression cassette does not contain a double stranded siRNA, and that the claims directed to a second amplification are proper dependent claims that recite additional steps. In view of these amendments, withdrawal of this rejection is requested.

Rejection Under 35 U.S.C. § 103(a)

The Examiner rejected claims 3-9, 17, 19-21, and 33-38 under 35 U.S.C. § 103(a) as being unpatentable over Lois-Caballe et al. (US 2003/0059944 A1) in view of MacFerrin et al., Lindermann et al., and Livache et al. The Examiner relies on the disclosure in Lois-Caballe of an RNA expression cassette having an RNA polymerase III promoter linked to a first RNA coding region and a first terminator sequence and a second RNA polymerase III promoter linked to a second RNA coding region and a second terminator. Figure 3, paragraphs 34 and 125. The Examiner also relies on Lois-Caballe's disclosure that generation of the viral construct can be accomplished by standard techniques of PCR. Paragraph 74. The Examiner acknowledges that Lois-Caballe does not explicitly teach that the siRNA expression cassette is produced by a PCR-based amplification method.

The Examiner relies on the disclosure in MacFerrin of expression-cassette-PCR (ECPCR), as shown in the bottom half of Figure 1. (Figure 1 caption reads, "in ECPCR, translational control sequences and restriction sites included in the primers are fused to the target sequence, thus providing an expression cassette ready for insertion into a bacterial

overexpression vector") "In ECPCR, sequences required for protein translation and restriction endonuclease digestion are incorporated into the primers, so that ECPCR of the target DNA results in synthesis of an expression cassette bearing all of the necessary information for cloning and translation in *E. coli*. (p. 1937) The Examiner relies on the general disclosure that "[t]he use of PCR to add new sequence information concomitant with amplification has found applicability in recombinant DNA technology, and the ECPCR protocol is representative of such methods. . . ." The length of noncomplementary sequences added during ECPCR is primarily limited not by PCR amplification, but by the size limitation of automated DNA synthesis (routinely >100 nucleotides); hence, the potential exists to incorporate additional sequence motifs (e.g. synthetic promoters or periplasmic signal sequences) during the ECPCR procedure." (p. 1940)

The Examiner relies on Lindermann for general disclosure that it is within the skill of the art to prepare primers capable of annealing to the primer binding sites. The Examiner further relies on Livache to teach that the principle of PCR is well known and use of PCR to produce double-stranded nucleic acid. (col. 5, 6)

According to the Examiner, it would have been obvious to one of ordinary skill in the art at the time the invention was made to make the expression cassette comprising the U6 promoter-siRNA sequence-termination signal sequence of Lois-Caballe by utilizing the ECPCR protocol of MacFerrin. One would have been motivated to do so with a reasonable expectation of success so as to produce an siRNA sequence expression construct in a more facile manner, because the ECPCR procedure of MacFerrin allows incorporation of additional nucleotide sequences into an expression cassette during PCR amplification, thereby enabling the production of a desired expression cassette without the need to perform cloning, and subcloning/ligation procedures. The Examiner further argues that

> **[s]ince the target-specific siRNA sequence is the variable that is 'added' into an expression cassette as 'new sequence information' when making expression cassettes encoding different siRNA sequences**, it would have been obvious to one of ordinary skill in the art to make a pair of PCR primers that hybridize with the constant nucleotide sequence, which is the

> human U6 promoter sequence recognized in the art to be suitable for transcribing siRNA sequences as taught by Lois-Caballe et al., whether or not one were to construct a cassette that synthesizes only one strand or both strands of a double-stranded siRNA sequence.

Office Action p. 10 (original emphasis; added emphasis in bold). Applicants traverse this rejection.

The references combined fail to teach or suggest each and every element of the claims. Specifically, each of the cited references taken together fail to teach or suggest 1) primers that flank a promoter sequence, one of which being 2) a primer that comprising a sequence which is complementary to a sequence encoding a sense sequence or antisense sequence of the double stranded siRNA molecule. MacFerrin teaches use of primers that flank different CD4 coding sequences (see p. 1938-40) and adding sequences required for protein translation (e.g. a promoter sequence) to these primers (see p. 1937, 1940). Livache discloses "primers containing promoter sequences for the amplification of the target sequence," and thus does not disclose the promoter sequence as the target sequence. Col. 6, lines 12-15 (emphasis added). Lindermann and Lois-Caballe only have general disclosures of PCR and primers. In fact, although Lois-Caballe discloses using PCR to make vector constructs, the PCR would appear to be designed to amplify the previously, fully synthesized expression constructs. Thus, primers would be designed for the entire pre-synthesized construct.

Even in view of MacFerrin, it was counter-intuitive to one skilled in the art to design a primer that flanks a promoter sequence, wherein the primer contains the desired "target" sequence. In fact, MacFerrin discloses that the ECPCR permits simultaneous 5' and 3' modifications of a coding sequence with flanking primers that incorporate sequences required for protein translation and restriction endonuclease digestion, and characterizes the ECPCR method as a "domainal analysis strategy." (p. 1937, abstract and first column). Furthermore, by beginning her analysis with the teachings of the present application (in bold above) in constructing her obviousness argument, without any similar teachings from the prior art, the

Examiner has succumbed to hindsight bias. None of the references teach or suggest adding to a primer a sequence that is complementary to a sequence encoding a sense or antisense sequence of a double stranded siRNA molecule, in which the primer is used to amplify a promoter sequence. None of the prior art teaches or suggests amplifying a promoter as set forth in the claimed subject matter.

In view of the above remarks, Applicants submit that the claimed invention is not obvious over the combination of Lois-Caballe et al., MacFerrin et al., Lindermann et al., and Livache et al. Withdrawal of this rejection is requested.

Conclusion

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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